Buccutite™ Fc Site-Specific Antibody Biotinylation Kit *Optimized for Labeling 1 mg Antibody*

Catalog number: 5570
Unit size: 2 Labelings (1 mg each)

Component	Storage	Amount (Cat No. 5570)
Component A: Fc Glycan Activator	Freeze (< -15 °C)	1 vial
Component B: Fc Reaction Buffer	Freeze (< -15 °C)	1 vial
Component C: Spin Desalting Column	Room temperature (10-25 °C)	2 x 2 mL columns
Component D: Buccutite-Fc-Biotin Crosslinker	Freeze (< -15 °C)	2 vials
Component E: DMSO	Freeze (< -15 °C)	100 µL
Component F: Fc Reaction Deactivator	Freeze (< -15 °C)	100 µL

OVERVIEW

Buccutite™ Fc Site-Specific Antibody Biotinylation Kit is designed for selective biotinylation of antibodies through Fc region-specific Buccutite™ conjugation chemistry. The Buccutite™ technology selectively targets accessible Fc-associated glycans for controlled antibody modification while minimizing labeling within antigen-binding regions. This site-specific approach helps preserve antibody binding activity and generates more uniform antibody conjugates compared to random lysine-labeling methods. The kit is optimized for labeling 1 mg antibody per reaction and offers a cost-effective approach for preparing Fc-specific biotinylated antibodies for downstream detection and affinity-based workflows. The resulting antibody-biotin conjugates are suitable for ELISA, immunohistochemistry, Western blotting, affinity purification, and other avidin/streptavidin-based detection workflows. Buccutite™ Fc site-specific conjugation technology provides a controlled alternative to random lysine-labeling approaches for generating more uniform antibody conjugates under mild reaction conditions.

AT A GLANCE

1. Prepare 500 µL antibody in PBS with concentration 2 mg/ml.
2. Add 50 µL Fc Reaction Buffer to antibody solution (500 µL) followed by addition of 50 µL Fc Glycan Activator.
3. Incubate at room temperature for one hour.
4. Add 20 µL Fc Reaction Deactivator into above solution, and incubate 10 min at RT.
5. Add 20 µL DMSO to Buccutite-Fc- Biotin Crosslinker to dissolve the powder, add it to Fc Activated Antibody. Incubate at room temperature for 60 minutes.
6. Purify the Buccutite-Fc- Biotin modified antibody with a Spin Desalting Column, collect the Fc-Biotin-modified antibody.
7. The Antibody Fc site activated with Biotin is ready to bind with streptavidin labels.

Important Notes:

1. Upon receipt, store Component A, B, D, E and F at -20°C. When stored properly, the kit should be stable for six months. Warm all the components and centrifuge the vials briefly before opening, and prepare the required solutions immediately before starting your conjugation.
2. The following SOP is an example for labeling 1mg of Anti-CD4 (SK3) monoclonal antibody (mouse IgG1) (500 µL with concentration 2 mg/ml). The antibody Fc modification efficiency is best if antibody concentration is above 5 mg/ml, and will be reduced if the antibody concentration is less than 2 mg/mL.
3. The reaction volume in each step can be adjusted proportionately based on the antibody volume.

PREPARATION OF WORKING SOLUTION

Antibody Working Solution

For labeling 1 mg antibody (assuming the target antibody concentration is 2 mg/mL), prepare 500 µL antibody solution in PBS.

Notes: 1. The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2 - 7.4. If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, or use Amicon Ultracel Membrane (30 kDa from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.

2. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.

Buccutite-Fc-Biotin Crosslinker Working Solution

Add 20 µL DMSO (Component E) into the vial of Buccutite-Fc-Biotin Crosslinker (Component D) to dissolve the powder.

SAMPLE EXPERIMENTAL PROTOCOL

Step 1: Fc Glycan Activation Reaction

1. Add 50 µL Fc Reaction Buffer (Component B) to antibody solution (500 µL), mix well by repeatedly pipetting for a few times.
2. Add 50 µL Fc Glycan Activator (Component A) into above antibody solution. (The total volume will be 600 µL).

   Note: If you have a different antibody concentration, please adjust Fc Glycan Activator and Fc Reaction Buffer volume proportionately.
3. Incubate the antibody Fc glycan activation reaction mixture at room temperature for one to two hours.

   Note: Incubation time longer than 2 hours is not recommended.
4. Add 20 µL Fc Reaction Deactivator (Component F) into above solution, and incubate for 10 min at RT.

Step 2: Modify Fc-Glycan Activated Antibody with Buccutite-Fc-Biotin Crosslinker

1. Add 20 µL of Buccutite-Fc-Biotin Crosslinker working solution into the Fc Glycan Activated Antibody solution, mix well by repeatedly pipetting for a few times.
2. Incubate at room temperature for 60 min.

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3. Purify Buccutite-Fc-MTA modified antibody with a Spin Desalting Column (Component C). See the **protocol to purify the reaction mixture with spin desalting column** below.

4. Antibody concentration can be measured with Nanodrop:
   Antibody Concentration (mg/ml) = A280 / 1.4

5. The Buccutite-Fc- Biotin modified antibody is ready to bind with streptavidin labels.

   Note: Buccutite-Fc- Biotin modified antibody could be stored at 4°C for several weeks when kept away from light.

Protocol to purify the reaction mixture with Spin Desalting Column:

1. Twist off the bottom closure of the desalting column (Component C) and loosen the cap. Place the column in a 15 mL collection tube.
2. Centrifuge the column at 1,000g for 2 minutes to remove the storage solution.
3. Remove the cap and slowly add 1 mL of 1X DPBS (not provided with the kit) to the column.
4. Centrifuge at 1,000g for 2 minutes, remove the buffer. Repeat this step for 3 additional times, discarding the buffer from the collection tube.
5. Place the column on a new 15 mL collection tube, and gently apply the sample into the center of the compact resin bed.
6. Centrifuge the column at 1,000g for 2 minutes to collect the sample.

DISCLAIMER

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